Hypotheses on Infertility in Captive White Rhinoceros (*Ceratotherium simum*): Thoughts on Normal and Abnormal Biology

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Abstract

Introduction

Ultrasonographic examinations of one multiparous 33-year-old female southern white rhinoceros (*Ceratotherium simum*) resulted in documentation of the animal's estrous cycle, elucidation of the timing of ovulation in relation to estrus, and ultasonographic evidence of endometritis and associated early embryonic death (Radcliffe, Czekala, and Osofsky, 1997).

The rhinoceros belongs to the order Perissodactyla, or "odd-toed" ungulates, which includes the horse and tapir. The equine species was used as a model for interpretation and evaluation of ultrasonographic information in this study, and this comparative approach has facilitated understanding of rhinoceros reproductive biology. A number of similarities were identified between the white rhinoceros female in this study (Radcliffe, Czekala, and Osofsky, 1997) and the documented reproductive biology of the mare (Ginther, 1992) as outlined below:

1) The formation of two ultrasonographically distinct luteal structures in an approximately even ratio.
2) The formation of anovulatory hemorrhagic follicles.
3) The identification of intrauterine fluid collections (IFCs) in late diestrus as an indication of endometritis.
4) The change in shape of preovulatory follicles from spherical to pear-shape in the 48 hours preceding estrus.
5) Similar estrous behaviors.

Proposed "Normal" White Rhino Reproductive Biology

Although the information presented here is preliminary, as it represents the study of one reproductively abnormal female, the findings provide important evidence corroborating behavioral observations of this species both in captivity (Rieches, personal communication) and in the wild (Owen-Smith, 1973). In addition, this study suggests that the longer 10 week cycles reported by some authors (Schwarzenberger et. al., 1994) utilizing fecal hormone assays alone may represent abnormal pathologic processes. As Ginther has stated (1995), a prolonged luteal phase currently cannot be adequately evaluated without ultrasound technology.

An interovulatory interval averaging 33 days (n=2) was identified during non-conceptive cycles, whereas longer periods of 73 and 78 days (n=2) were identified in both cases where early embryonic death (EED) was documented via ultrasonography (Figure 1). Obviously, more intensive study is needed to confirm these findings. However, significant technical conclusions can be drawn from this study, especially since the ultrasonographic and fecal hormone evaluations encompassed a year-long period and a number of duplicated reproductive events (Radcliffe, Czekala, and Osofsky, 1997).

Proposed Hypotheses Regarding Captive White Rhino Infertility

Hypothesis I : EED and luteal persistence to describe observed 10 week cycles

Case Study

Both pregnancies monitored in this female in 1995 were lost by Day 28 post ovulation, with collapse of the embryonic vesicle documented via ultrasound. Ultrasonographic evidence of endometritis was observed in this female and was characterized by small quantities of anechoic intrauterine fluid collections (Figure 2; 5-20 mm in diameter) in late diestrus (n=4, mean day observed was 20.5 days postovulation, with a range of 18- 24 days). In mares, a strong correlation has been shown between the identification of IFCs in late diestrus and impending EED (Ginther, 1992), and both IFCs and EED were more extensive in old than young mares (Carnevale and Ginther, 1992). The founder white rhinoceros individuals are old; advancing age in this population is likely to be a significant factor reducing fertility. Fecal samples collected at the time of ultrasound were evaluated via radioimmunoassay for progesterone metabolites. In both cases of EED, the progestin levels remained elevated and the ultrasonographically identifiable corpus luteum (CL) persisted for a period of 10-11 weeks (Figure 1).

In the horse, IFCs are thought to represent an inflammatory exudate or the uterine response to irritation (Ginther, 1992). Endometritis (as represented by IFCs) has been shown to induce EED through two different mechanisms:

> *Mechanism 1*) The inflammatory exudate can induce luteolysis through stimulation of release of uterine PGF2α. With luteolysis and the associated decline in progesterone, embryo fixation fails due to inadequate uterine tone (Ginther, 1992). Most embryos lost via this mechanism in the mare are eliminated by Day 11 (Ginther, 1992), and thus not ultrasonographically detected.
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> *Mechanism 2*) The inflammatory products or pathogens can produce direct toxic effects on the embryo (Ball, 1993). Ball (1993) reported that there is an increased metabolic demand on the conceptus beyond Day 28.

In the case study described here the observed persistence of the CL, as evidenced by its continued ultrasonographic detection beyond the time of embryo loss and the prolonged elevation of fecal progestins (Figure 1), supports EED by mechanism 2. The timing of EED by Day 28 also supports a toxic cause by mechanism 2 as compared to the earlier EED often observed with mechanism 1. Thus, the 10 week periods observed elsewhere (Schwarzenberger et. al., 1994) could be the result of an age-related deterioration in the female white rhino's uterine environment.

Hypothesis II : Hemorrhagic follicles as a cause of irregular cycle length

The identification of anovulatory hemorrhagic follicles (HAFs) has been described in the horse (Ginther, 1992) and the rhinoceros (Radcliffe, Czekala, and Osofsky, 1997). Even in the domestic equine species where intensive reproductive research has been ongoing for years, hemorrhagic follicles remain little understood.

Hemorrhagic follicles are large anovulatory follicles that fill with blood (Figure 3). The resulting structure typically measures 60 mm or more in diameter in both the horse and the rhino, and is characterized by intraluminal fibrinous bands that are observed to quiver upon ballottement during ultrasonographic examination. In the mare, swirling or quivering of hemorrhagic contents for two days can be used to diagnose anovulation (Ginther, 1995), and thus differentiate between the anovulatory HAF and the ovulatory corpus hemorrhagicum. Consequently, HAFs have been associated with prolonged interovulatory intervals since these hemorrhagic structures were presumed to be anovulatory (Ginther, 1992).

In our studies at Fossil Rim, we have identified structures analogous to HAFs in both the white rhinoceros and black rhinoceros species. The formation of these structures was documented on several occasions in the black rhinoceros and was associated with irregular cyclic activity in one female and significant elevations in fecal progestins (>800 ng/g) in another female (Radcliffe, Czekala, and Patton, 1997 unpublished data). These hemorrhagic structures have appeared to luteinize on some occasions based on changing ultrasonographic appearance (Figure 3), supporting the observed elevations in progestins. HAFs should not be confused with true cystic follicles, persistent fluid- filled ovarian structures described in species such as cattle, and considered pathologic (Arthur et. al., 1996). As in the horse (Ginther, 1992; 1995), all HAFs observed in our ultrasound studies of the rhinoceros have been dynamic structures that regress in size and disappear over the course of 3-4 weeks. In the mare, hemorrhagic follicles are considered irregular, but not pathologic structures, and the syndrome of "cystic ovaries" has not been documented as an entity (Ginther, 1992).

Hypothesis III : Seasonality, stress or management factors as causes of infertility

Unlike in the horse, there is no indication for true seasonal anestrus in the rhinoceros (since breeding and production of offspring has been documented in all times of the year). However, a number of factors may affect the fertility of the white rhinoceros in captivity. In our facility, management factors may play an important role in the observed infertility during the winter months. The rhinos are moved to a barn during cold times of the year (November through March), and this movement, dependent on weather conditions, always involves separation of males and females and likely increased stress. In Radcliffe, Czekala, and Osofsky (1997), the observed lack of ovulation and low levels of progestin during the months of January through March may indicate that the stress of confinement or separation, for example, adversely affected fertility.

Conclusions

It is unlikely that any one factor is responsible for the observed infertility in the captive population of white rhinoceros, but instead, a combination of behavioral, management, and reproductive factors may be working in consort to create the problem. Obviously more research is needed to document the normal reproductive biology of the rhinoceros before meaningful attempts can be made to alter or stimulate reproductive function in these endangered mammals. Although time may be running out for many of the founder animals due to advancing age and the adverse effects of long periods of reproductive quiescence, long-term management of any viable captive population of this species mandates that we better understand their normal biology. This report will hopefully stimulate further research in that direction.

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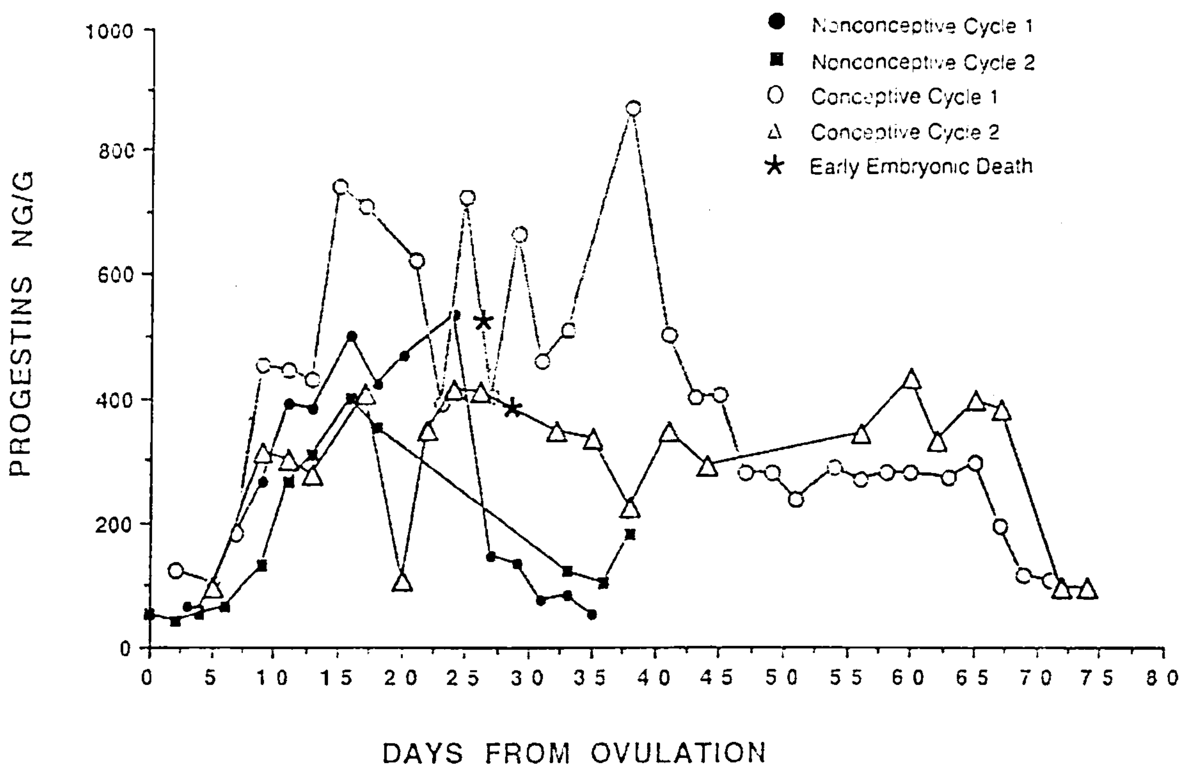

Figure 1. A comparison of fecal progestin concentrations from two nonconceptive cycles (closed symbols) and two conceptive periods in which early embryonic death (EED) was observed (open symbols).

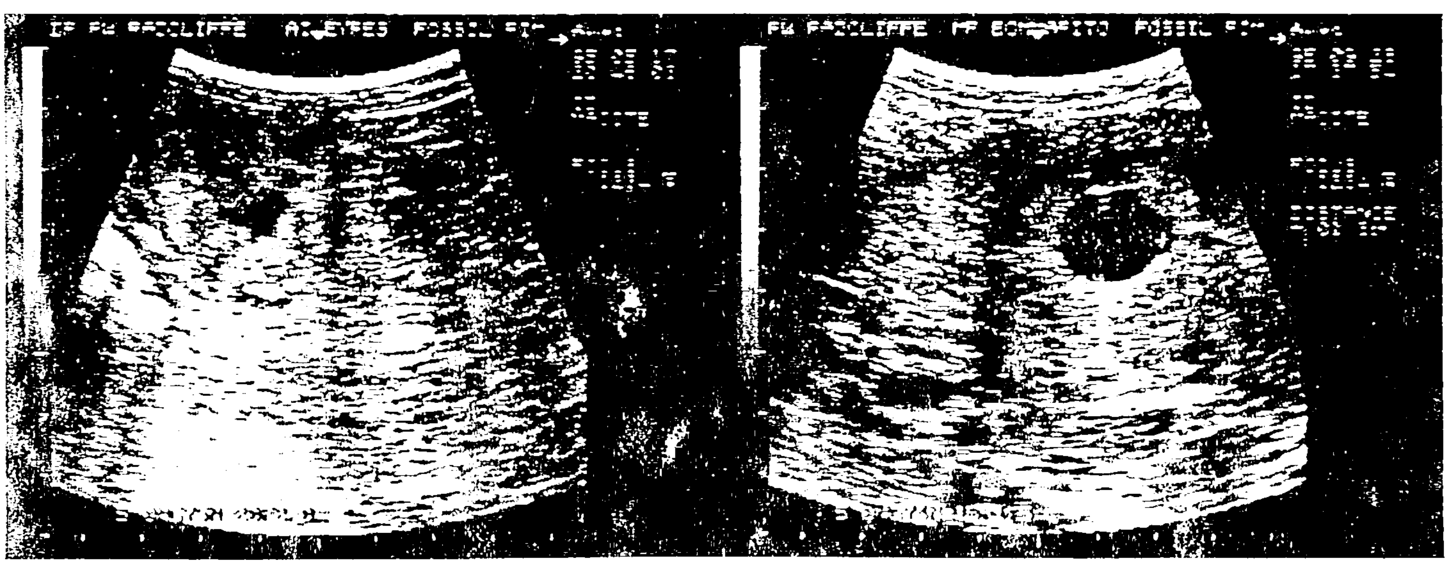

Figure 2. Intrauterine fluid collections (IFC) observed in late diestrus (left) and surrounding a 19-day embryonic vesicle (right), providing strong evidence for impending early embryonic death (EED) [adapted from Radcliffe, Czekala, and Osofsky, 1997].

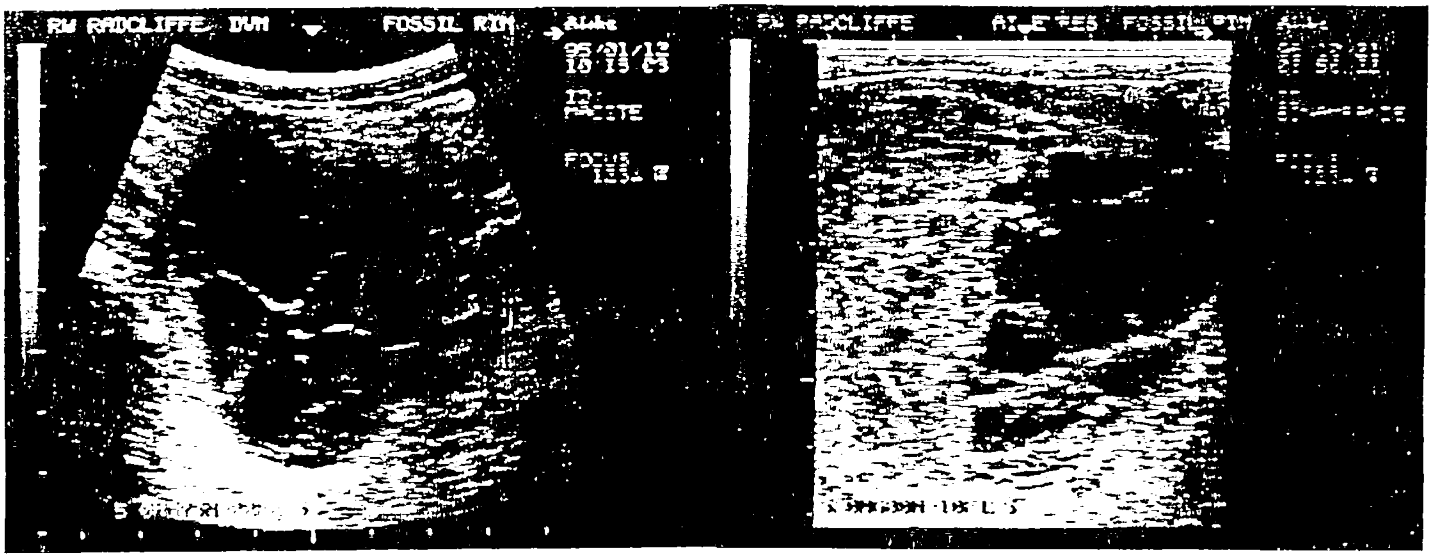

Figure 3. Hemorrhagic follicles (HAF) identified in the white rhinoceros (left; adapted from Radcliffe, Czekala, Osofsky, 1997) and the black rhinoceros (right; note apparent lutenization). These follicles are considered anovulatory but not pathologic structures in the horse, and have been associated with irregular cycle lengths in the rhinoceros.